

FINAL REPORT

INDUCTION OF FRUITING IN AN UNIDENTIFIED BASIDIOMYCETE,
A MYCANGIAL SYMBIONT OF THE SOUTHERN PINE BEETLE

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INTRODUCTION

Two fungi have consistently been isolated from the mycangium of the southern pine beetle (SPB) (Barras and Perry 1972). These fungi are important to the successful development of the insect (Barras 1973). One has been identified as a *Ceratocystis* species with a *Sporothrix* imperfect stage (Barras and Taylor 1973). The other (SJB 122) has presented problems in its identification because of its failure to produce fruiting structures. SJB 122 exists in two forms. When squeezed from the mycangium, the ambrosial or yeast-like form is apparent. It proliferates in the mycangium as globular, chlamydosporous, short chained hyphae. When inoculated onto media, the fungus has the characteristics of a slow growing filamentous fungus. It produces a brown exudate, clamp connections, and sterile mycelia. It has never fruited in culture. It was concluded that this organism is a basidiomycete on the basis of the production of clamp connections which have never been observed in fungi other than Basidiomycetes.

The purpose of this study was to induce or stimulate the fungus to form fruiting bodies or spores in response to mechanical injury. The fungus could then be identified on the basis of its fruiting structures and spores.

METHODS AND MATERIALS

SJB 122 was isolated from the mycangia of SPB and cultured on a basic medium (BM) containing 20.0 g dextrose, 2.0 g peptone, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g. K_2HPO_4 , 120 mg thiamine HCl and 20.0 g agar per liter of distilled water. Four plates of BM were inoculated to serve as transfer inoculum. The plates were incubated at 25°C and allowed to grow for 2 weeks or until colonies had attained a diameter of at least 5 cm.

Three modifications of the basic medium were utilized consisting of (1) only 1 g peptone ($\frac{1}{2}$ PBM), (2) the addition of 1.0 g yeast extract (BMYE), and (3) dl-asparagine (BMA) substituted for peptone. Media were sterilized by autoclaving 20 minutes at 121°C. Dextrose was autoclaved separately to minimize caramelization.

In one experiment hyphae were cut with a sterile cork borer. The experiment consisted of 4 replicates of each of the 4 media (BM, $\frac{1}{2}$ PBM, BMYE, BMA). The inoculum was a 10 mm plug from a 2-week old culture of the fungus grown on each of the 4 media. The 10 mm plug was placed inside a previously removed 15 mm plug of the test medium. This provided an air space of 5 mm around the inoculum and allowed a minimal amount of nutrition to be obtained from the original medium (Leonard and Dick 1973). The plates were incubated for two weeks at 25°C to allow the relatively slow-growing mycangial fungus to attain a diameter of at least 4 cm.

The fungus on each of the 4 media was also mechanically injured by cutting with a sterile scapel. Two cuts were made at right angles to each other from center to edge of each colony. One-fourth of the colony was removed exposing the cut edges. The plates were reincubated and observed every 3 days for the production of fruiting bodies along the cut edges.

RESULTS AND DISCUSSION

Colony characteristics varied on the four media. Rate of growth, type of aerial mycelia, and colony color and texture were affected.

On BM, the colony attained a diameter of 72 mm. A dark brown exudate diffused throughout the plate discoloring the medium even where there were no mycelia. Near the center of the colony white, cottony aerial mycelia were produced. Along the outer edge of the colony, aerial mycelia were brown in color. Gelatinous pustules were produced over the surface of the medium.

More growth was produced on BMYE than on BM, and the colony attained a diameter of 80 mm. The agar surface was covered with brown aerial mycelia; white mycelia were sparse. The medium was stained a darker brown than on BM, but no gelatinous pustules were formed.

The colony on $\frac{1}{2}$ PBM attained a diameter of 70 mm. The surface of the colony was depressed, and only scanty aerial mycelia were produced.

BMA produced the least amount of growth with a colony having a diameter of 53 mm. Aerial mycelia were cottony and beige in color. Gelatinous pustules were formed only on the original inoculation plug. The surface of the colony was depressed and sectoried. A tan exudate formed on the surface of the media and the medium itself was stained light brown.

Observations of hyphae cut either with a scapel or cork borer showed that SJB 122 did not produce fruiting structures in response to mechanical injury.

No reproductive structures were found on any of the media, the cut edge of the fungi, or on fungi growing in and over the 5 mm space. On BMA the fungus showed a form of growth along the cut edge of the medium that was previously unknown to occur on artificial media. The fungus no longer grew in its sterile mycelial state, but produced a proliferating, translucent mass of yeast-like cells similar to the growth form found in the mycangium. These yeast-like cells were multinucleate, presumably haploid, and assumed to be the product of meiosis.

When transferred to malt extract agar, the cells germinated into hyphae, and nuclear migration occurred to form a monokaryotic mycelium. As the culture aged, compatible hyphae fused forming a dikaryotic mycelium with clamp-connections. These morphological aspects of the fungus suggest a relationship to the heterobasidiomycetous yeasts (Fell 1970).

It is possible that the spores picked up by the beetle in its mycangium are nourished by nutrient deficient secretions which propagate only the yeast

form of the fungus. When transferred to any nutrient rich media, these then germinate to form the dikaryotic mycelium bearing clamp connections with no obvious reproductive structure.

Except on BMA, no response to injury occurred. The fungus continued to produce sterile mycelia on all media and in both tests.

This study is closed.

LITERATURE CITED

- Barras, S. J. 1973. Reduction of progeny and development in the southern pine beetle following removal of symbiotic fungi. *Can. Entomol.* 105: 1295-1299.
- Barras, S. J. and T. Perry. 1972. Fungal symbionts in the prothoracic mycangium of *Dendroctonus frontalis* (Coleoptera:Scolytidae). *Z. ang. Entomol.* 71:95-104.
- Barras, S. J. and J. J. Taylor. 1973. Varietal *Ceratocystis minor* identified from mycangium of *Dendroctonus frontalis*. *Mycopathologia et Mycologia applicata* 50:293-305.
- Fell, J. W. 1970. Yeasts with Heterobasidiomycetes life cycles. IN Recent trends in yeast research. Ed. D. A. Ahern. Georgia State University Press. Ga. State Univ., Atlanta, pp. 49-66.
- Leonard, T. J. and S. Dick. 1973. Induction of haploid fruiting by mechanical injury in *Schizophyllum commune*. *Mycologia* 65:809-822,